Invited lecture/Scientific contribution

Automated Quantification of Microplastics – Challenges and Opportunities

Prosenc F[1,*], Van de Velde N[2], Jerman I[2], Langus J[3]

1. University of Ljubljana, Faculty of Health Sciences, Ljubljana, Slovenia
2. National Institute of Chemistry, Ljubljana, Slovenia
3. C3M, d.o.o., Ljubljana, Slovenia

* Correspondence: Franja Prosenc; franja.prosenc@zf.uni-lj.si

Abstract:
Plastics are an important material with widespread applications. However, their widespread use and poor end-of-life management have led to their extensive environmental pollution. They can be found in oceans, terrestrial ecosystems, and even remote corners of the Earth. Current methods for microplastic quantification and identification require big investments and highly trained personnel to operate the analytical equipment. In this paper, we propose an algorithm-based method for the quantification of microplastics in soil and organic fertilisers. The method is based on image analysis of a thinly spread sample that was heated until microplastics has visually melted. The algorithm-based method was validated with Focal plane array detector-based micro-Fourier-transform infrared imaging (FPA-μFTIR), frequently used in microplastic characterisation. Herein, we present the preliminary results of an ongoing study. In a compost sample, five particles were detected with FPA-μFTIR, whereas the algorithm detected eight. The algorithm has difficulties recognising elongated or oddly shaped particles. These were identified as several particles which led to overestimating the number of microplastic particles in the investigated sample. We will continue with further development of the computer algorithm by using a training set of images which will be quantified using different methods (visual detection by a human operator, FPA-μFTIR). This growing training set will enable us to incorporate machine learning algorithms (neural networks) in the development of a more reliable particle detection algorithm. We expect that environmental monitoring of microplastics will be required under future legislation, therefore the development of cheap, user-friendly solutions is crucial.

Keywords: Machine learning; Algorithm; Infrared spectroscopy; Soil contamination; Organic fertilisers; Compost

Citation: Prosenc F, Van de Velde N, Jerman I, Langus J. Automated Quantification of Microplastics – Challenges and Opportunities. Proceedings of Socratic Lectures. **2022**, 7: 83-88. https://doi.org/10.55295/PSL.2022.D12

1. Introduction

1.1. The need for automation

Plastics are a widespread material with critical applications across different sectors. However, their increasing production rates and poor end-of-life management are causing widespread pollution (PlasticsEurope, 2021; Geyer et al., 2017). Plastic contamination has now been found in every ecosystem, from oceans to land (Chae and An, 2017; Zhou et al., 2020), and even in the most remote corners of the world (Bergmann et al., 2019; Materić et al., 2022).

Currently, microplastics are isolated from environmental samples and quantified predominantly manually. Despite the numerous reports on the unsuitability of manual identification and sorting of microplastic particles due to human error and bias tendency, this is still the most widely used method, due to its simplicity and wide availability (Silva et al., 2018).

Due to the scale of plastic pollution and the public attention, this has received, we expect that microplastic monitoring will be mandatory under future legislation. For this, user-friendly automated quantification solutions will be of paramount importance.

1.2. The current state-of-the-art of automated quantification

In recent years, the development of commercial solutions for automated microplastic analysis, especially in the field of infrared spectroscopy has progressed. Instruments, such as Bruker Lumos II Fourier-transform infrared (FTIR) microscope and Agilent laser-direct infrared (LDIR) Chemical imaging system, are capable of scanning samples directly on a filter or a microscope slide and recording infrared spectra of (microplastic) particles. Spectra are then matched to reference libraries for identification. These instruments offer sophisticated analysis; however, they require a big investment and highly trained personnel to operate them.

Another solution for automated quantification includes custom-built hardware, e.g., fluorescent microscope attachment with blue LEDs for smartphones. Microplastic particles are stained on a filter with Nile Red, which is then excited by the blue LEDs which causes stained microplastics to fluoresce. Quantification is done using MATLAB algorithms (Leonard et al., 2022).

Other research focuses on quantification with near-infrared hyperspectral imaging (NIR-HIS). Hyperspectral cameras scan the sample and obtain a near-infrared spectrum of each pixel on the picture. This enables the recognition of microplastic particles directly on a filter or microscope slide. Quantification is then done manually or with computer algorithms. So far, this method has been demonstrated on artificially prepared (spiked) samples of water and soil (Piarulli et al., 2020; Shan et al., 2018).

1.3. Our aim

Timely quantification of microplastic numbers is critical for predicting the ecosystems' health. However, current methods for microplastic quantification are inadequate to handle high-frequency sample quantification. We, therefore, aim to develop an automated widely accessible analytical tool using image processing with a machine learning algorithm for the quantification of microplastic particles. In this method, there is no need for specialised hardware. A regular camera or smartphone camera can be used to record sample photos. The work is still in progress, and we, herein present preliminary results, challenges to overcome and opportunities for achieving our aim.

2. Methods

2.1. Experimental design

The objective of the study was to compare two methods for microplastic quantification: (1) Focal plane array detector-based micro-Fourier-transform infrared imaging (FPA-μFTIR), frequently used in microplastic characterisation, and (2) algorithm-based image processing, which showed the potential to be more time-efficient and user friendly.

Samples were extracted from soil and compost by density separation with saturated $ZnCl_2$ solution as reported by Prosenc et al., 2021. In short, 10 g of sample was placed in a 50 mL centrifuge tube, and $ZnCl_2$ (density 1.6 g cm^{-3}) was added to the 50 mL label. The tube was then shaken vigorously for 30 sec. Samples were centrifuged for 15 min at 6000 rpm. The supernatant was filtered with a 45 µm glass fibre filter. The filters were dried at 60 ºC and microplastics and the remaining debris were collected in a glass vial. A portion of the extract (1/10th of mass) was spread out to a 1 cm^2 area on a silicon wafer.

2.2. *Infrared spectroscopy for detection of microplastics*

First, the samples were analysed with a Hyperion 3000 FTIR microscope (Bruker Optics GmbH, Germany) equipped with a focal plane array (FPA) detector with 128 × 128 detector elements. Measurements were performed in reflection mode using a 15× IR objective with a 350 x 350 µm field of view. The signal coming from the 128 x 128 elements was averaged into 8 x 8 pixels, resulting in a final spatial resolution of 43.75 µm per pixel. All spectra were recorded using 16 accumulations at a resolution of 8 cm^{-1} between 3850 and 900 cm^{-1}. Spectra processing was done in the OPUS 8.5 software (Bruker Optics).

2.3. *Algorithm-based recognition of microplastics*

After the FTIR analysis, photos of samples were recorded with a camera (Cyber-shot DSC-RX100 II, Sony, Japan). Samples were heated on a hotplate (Schott Ceran Top Line 2000, Rommelsbacher, Germany) while recording the temperature until microplastic particles were visibly melted. During the heating of the samples, a series of high-resolution snapshots were taken in continuous mode (1 per second).

The photos were then processed with a computer algorithm developed to streamline the detection of microplastic particles during the sample heating process. This algorithm uses snapshots of the melting process and calculates differences between subsequent snapshots. Small differences must be neglected due to the slight movement of particles during heating, but large differences indicate the presence of microplastic particles, since these particles melt during the heating process, changing both, shape and colour.

2. Results

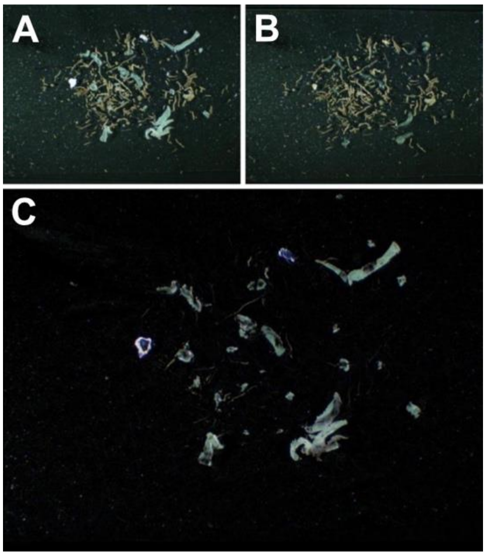

Figure 1. Processing of samples and creating a probability mask for potential microplastic particles. A – a photograph of a soil sample, containing microplastic particles; B – sample after heating; C – a probability mask with unchanged particles filtered out.

2.1. The principle

The computer algorithm developed for automated microplastic quantification first calculates differences between sample pictures before and after heating. When heated, thermoplastic microplastics melt and change shape and colour, while other particles remain unchanged. This creates a mask of the probability of microplastic particle presence. The probability mask is further processed to determine possible microplastic particle locations on the image and filters out particles that did not change shape and colour during the heating process (**Figure 1**). During the filtering step, certain assumptions on expected microplastic particle shape and size are made to minimise the number of false-positive identifications.

2.2. Quantification of microplastics in a compost sample

The computer algorithm was trialled for automated microplastic quantification in a compost sample that underwent microplastic extraction. It detected eight potential microplastic particles. The algorithm correctly located three microplastic particles (Figure 2C), however, the detection at two of these was multiple, e.g., three particles recognised in place of one. The algorithm also has difficulties with elongated or oddly shaped microplastic particles. These are usually identified as several particles which could lead to overestimating the number of microplastic particles in the investigated sample. Another detection was a non-plastic particle (**Figure 2**, red arrow). This particle moved during the heating process due to convection and was therefore recognised as a particle that changed shape by the algorithm. This could potentially be avoided by using a slower heating process and further development of the computer algorithm. One larger particle (bottom left) and several smaller ones (centre) in this sample were not recognised by the algorithm, as can be seen from the validation step (**Figure 3**).

Figure 2. Subsequent images of a compost sample, spread across a 1 cm² area. A – before heating, B – after heating, C – algorithm processed.

The same sample that was processed with the algorithm, was validated with FPA-µFTIR, a frequently used analytical technique in microplastic identification and characterisation (Primpke et al., 2017). With this method, we mapped four larger particles (**Figure 3A, B**, and **D**) and possibly several smaller ones **(Figure 3C**).

Integration at different infrared (IR) regions (at different wavenumbers) reveals particles absorbing in those IR regions. In this sample, integrations in the following regions 1405-1490 cm^{-1}, 1455-1490 cm^{-1}, 2820-2970 cm^{-1}, gave a good signal for four larger particles that were identified as polyethylene (PE) (**Figure 4A, B**, and **D**). The several smaller particles (integrated between 1715 and 1760 cm^{-1}) were identified as polyester (PES) (**Figure 4C**). These could be several smaller particles or a cluster of fibres spanning across several focal planes, where only parts of fibres were in focus and gave a good enough IR signal. We found that the optimal IR signal is usually acquired at or just below the visual focus. This can be problematic with bigger particles because all particles in the sample might not be focused at the optimal focal plane and a compromise has to be made to acquire a signal for as many particles as possible.

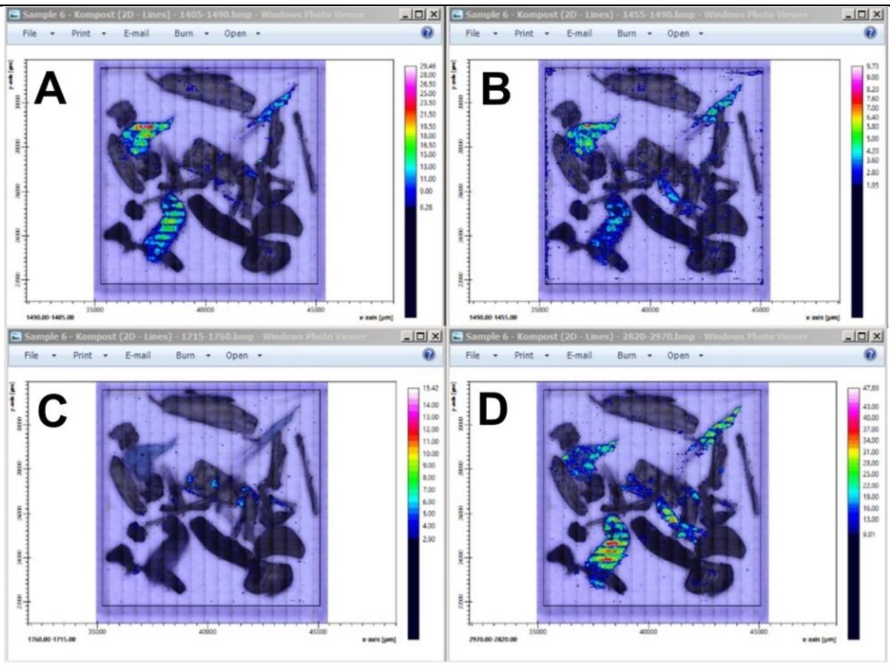

Figure 3. Infrared signal at different wavenumber integrations. Different plastic polymers give signals at different wavenumber integrations. A – integration at 1405-1490 cm^{-1}, B – integration at 1455-1490 cm^{-1}, C – integration at 1715 and 1760 cm^{-1}, and D – integration at 2820-2970 cm^{-1}.

The detection was matched in three out of five microplastic particles between the two methods (neglecting multiple detections of the same particle by the algorithm and assuming that the cluster of smaller PES particles detected by FPA-µFTIR was one particle). Our future work will focus on the further development of the computer algorithm. Currently implemented detection based on calculating colour and shape differences does not give optimal results for elongated or oddly shaped particles and particles that are stained and subsequently do not change colour during the heating process. We are constantly building a base of samples, which are quantified using different methods (visual detection by a human operator, FPA-µFTIR). This growing training set will enable us to incorporate machine learning algorithms (neural networks) in the development of a more reliable particle detection algorithm.

3. Discussion

This paper presents preliminary results of ongoing research, and the principles used in the development of a method for automated quantification of microplastics. This method has similar challenges to methods reported in the introduction. When working with solid matrices, especially rich in organic matter, such as soil and organic fertilisers, the presence of some residual debris is unavoidable. It can be significantly reduced by oxidation and digestion procedures (Hurley et al., 2018), but some remains in the extract. In methods relying on visual techniques, particles obscuring microplastic can be an issue, therefore, a lot of care should be taken when preparing the sample, e.g., spreading it out thinly, ensuring that particles are not obscuring other particles, etc.

Another issue with visual techniques is the challenges related to image capturing. This goes for the algorithm-based method as well as the FPA-µFITR method. The big particles are spreading across several focal planes, which makes obtaining a good focus and therefore a strong and even infrared signal with FPA-µFTIR, difficult. When capturing photographs for the algorithm processing, high-resolution images, and constant camera settings during the heating process (F-stop, exposure, ISO value) are crucial.

The limitation of this and other heating-related methods (e.g., pyrolysis GC-MS) is that they are not appropriate for thermosets. These are plastic polymers that become set in their physical and chemical properties after initial heat treatment but cannot be remoulded or heated after the initial forming.

Examples include epoxy, polyurethane (PU), and silicone, amongst others. However, the most commonly found microplastics in the environment are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET), all belonging to the thermoplastics class (Nerland et al., 2014), therefore, this method could importantly contribute to the microplastic monitoring efforts.

Despite the challenges, this method also offers an opportunity to couple fast, automated, and user-friendly quantification of microplastics with potential identification of microplastic polymers. During the heating process, the temperature of the hotplate is recorded. We will couple the recorded temperature to the melted plastic particles in a time sequence. Due to the different melting temperatures of polymers, we will be able to estimate the polymer composition of different particles.

The challenges in automated, affordable, and user-friendly quantification of microplastics are vast. However, microplastics research, including their analysis, is a fast-paced field with new solutions emerging regularly. We expect that microplastic monitoring will be regulated in future legislation, therefore, the availability of high-throughput reliable methods is essential.

Funding: This research was funded by the Slovenian Research Agency grant Z2-2643, core funding P3-0388, and C3M d.o.o.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bergmann M, Mützel S, Primpke S, et al. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. Sci. Adv. 2019; 5: eaax1157. DOI: 10.1126/sciadv.aax1157.
2. Chae Y, An YJ. Effects of micro- and nanoplastics on aquatic ecosystems: Current research trends and perspectives. Mar. Pollut. Bull. 2017; 124: 624-632. DOI: 10.1016/j.marpolbul.2017.01.070.
3. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Sci. Adv. 2017; 3: e1700782. DOI: 10.1126/sciadv.1700782.
4. Hurley RR, Lusher AL, Olsen M, Nizzetto L. Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environ. Sci. Technol. 2018; 52: 7409-7417. DOI: 10.1021/acs.est.8b01517.
5. Leonard J, Koydemir HC, Koutnik VS et al. Smartphone-enabled rapid quantification of microplastics. J. Hazard. Mater. Let. 2022; 3:100052. DOI: 10.1016/j.hazl.2022.100052.
6. Materić D, Kjær HA, Vallelonga P, et al. Nanoplastics measurements in Northern and Southern polar ice. Environ. Res. 2022; 208: 112741. DOI: 10.1016/j.envres.2022.112741.
7. Nerland IL, Halsband C, Allan I, Thomas KV. Microplastics in marine environments: Occurrence, distribution and effect [Report]. 2014; Accessed: 30.4.2022. Available from: https://www.miljodirektoratet.no/globalassets/publikasjoner/M319/M319.pdf.bergaman
8. Piarulli S, Sciutto G, Oliveri P, Malegori C et al. Rapid and direct detection of small microplastics in aquatic samples by a new near infrared hyperspectral imaging (NIR-HSI) method. Chemosphere. 2020; 260: 127655. DOI: 10.1016/j.chemosphere.2020.127655.
9. PlasticsEurope (2021), Plastics – the Facts 2021; An analysis of European plastics production, demand and waste data [Report]. Accessed: 27.1.2022. Available from: https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/.
10. Primpke S, Lorenz C, Rascher-Friesenhausenbc R, Gerdtsa G. An automated approach for microplastics analysis using focal plane array (FPA) FTIR microscopy and image analysis. Anal. Methods. 2017; 9: 1499. DOI: 10.1039/c6ay02476a.
11. Prosenc F, Leban P, Šunta U, Bavcon Kralj M. Extraction and Identification of a Wide Range of Microplastic Polymers in Soil and Compost. Polymers. 2021; 13: 4069. DOI: 10.3390/polym13234069.
12. Shan J, Zhao JZ, Liu L, Zhang Y et al. A novel way to rapidly monitor microplastics in soil by hyperspectral imaging technology and chemometrics. Environ. Pollut. 2018; 238:121e129. DOI: 10.1016/j.envpol.2018.03.026.
13. Silva AB, Bastos AS, Justino CIL, et al. Microplastics in the environment: Challenges in analytical chemistry - A review. Anal. Chim. Acta. 2018; 1017: 1-19. DOI: 10.1016/j.aca.2018.02.043.
14. Zhou Y, Wang J, Zou M, et al. Microplastics in soils: A review of methods, occurrence, fate, transport, ecological and environmental risks. Sci. Total. Environ. 2020; 748: 141368. DOI: 10.1016/j.scitotenv.2020.141368.